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Attorney Reference No. 245-62504
PATENT

EXPRESS MAIL LABEL NO. EV053213824US
DATE OF DEPOSIT: March 29, 2002

JC05 Rec'd PCT/PTO 29 MAR 2002

FORM PTO-1390 U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. § 371

ATTORNEY'S DOCKET NUMBER	
245-62504	
U.S. APPLICATION NO. (If known, see 37 C.F.R. § 1.5)	
10/089428	

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US00/23713	29 August 2000	1 October 1999

TITLE OF INVENTION
LOW ERUCIC ACID MEADOWFOAM PLANTS AND OILS PRODUCED FROM THEM

APPLICANT(S) FOR DO/EO/US
Steven J. Knapp and Jimmie M. Crane

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. § 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. § 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. § 371(f) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. § 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. § 371(c)(2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ has been transmitted by the International Bureau.
   c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. § 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. § 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. § 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. § 371(c)(4)) (unsigned).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. § 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 C.F.R. §§ 1.97 and 1.98, accompanied by 28 documents.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 C.F.R. §§ 3.28 and 3.31 and the Recordal fee of $40.00 is included.
13. ☒ A **FIRST** preliminary amendment, with attached abstract on a separate page.
    ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information:
    ☒ Written Opinion dated 11 October 2001.
    ☒ A second Written Opinion dated 15 October 2001.
    ☒ International Search Report dated 18 December 2000.

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17. ☒ The following fees are submitted:

BASIC NATIONAL FEE (37 C.F.R. §§ 1.492(a)(1)-(5)):

Neither International Preliminary Examination fee (37 C.F.R. § 1.482) nor International Search fee (37 C.F.R. § 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1,040.00**

International Preliminary Examination fee (37 C.F.R. § 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO..... **$890.00**

International Preliminary Examination fee (37 C.F.R. § 1.482) not paid to USPTO but International Search fee (37 C.F.R. § 1.445(a)(2)) paid to USPTO as an International Searching Authority **$740.00**

International Preliminary Examination fee paid to USPTO (37 C.F.R. § 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4)..... **$710.00**

International Preliminary Examination fee paid to USPTO (37 C.F.R. § 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4)..... **$100.00**

				CALCULATIONS (PTO USE ONLY)
ENTER APPROPRIATE BASIC FEE AMOUNT =				$ 710.00
Surcharge of **$130.00** for furnishing the signed oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 C.F.R. § 1.492(e)).				$
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	27 - 20 =	7	x $18.00	$ 126.00
Independent Claims	6 - 3 =	3	x $84.00	$ 252.00
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $280.00	$
TOTAL OF ABOVE CALCULATIONS =				$ 1,088.00
☒ Reduction of 1/2 for filing by small entity. Small entity status is claimed for this application.				$
SUBTOTAL =				$ 544.00
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 Months from the earliest claimed priority date (37 C.F.R. §§ 1.492(f)). +				$
TOTAL NATIONAL FEE =				$ 544.00
Fee for recording the enclosed assignment (37 C.F.R. § 1.21(h)). The assignment must be Accompanied by an appropriate cover sheet (37 C.F.R. §§ 3.28, 3.31). **$40.00** per property. +				$
TOTAL FEES ENCLOSED =				$ 544.00
				REFUND → $
				CHARGE → $

a. ☒ A check in the amount of $544.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of $ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Director is hereby authorized to charge any additional fees that may be required, or credit any overpayment, to Deposit Account No. 02-4550. A duplicate copy of this sheet is enclosed.

d. ☒ Please return the enclosed postcard to confirm that the items listed above have been received.

NOTE: Where an appropriate time limit under 37 C.F.R. § 1.494 or § 1.495 has not been met, a petition to revive (37 C.F.R. § 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

KLARQUIST SPARKMAN, LLP
One World Trade Center, Suite 1600
121 S.W. Salmon Street
Portland, OR 97204-2988

SIGNATURE
Tanya M. Harding, Ph.D.
NAME

42,630
REGISTRATION NUMBER

cc: Docketing

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Knapp and Crane

Art Unit: Not yet assigned

Application No.: Not yet assigned

Filed: Herewith

For: LOW ERUCIC ACID MEADOWFOAM
PLANTS AND OILS PRODUCED FROM THEM

Examiner: Not yet assigned

Date: March 29, 2002

BOX PCT
COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

PRELIMINARY AMENDMENT

In the specification, please add the following paragraph at the top of page 1, immediately after the title:

--REFERENCE TO RELATED CASES

This application claims priority to co-pending International Application Number PCT/US00/23713, filed August 29, 2000, which claims the benefit of U.S. Provisional Application Number 60/157,258, filed October 1, 1999, and U.S. Provisional Application Number 60/157,256, filed October 1, 1999. --

Please insert the following abstract with title (which is also submitted on a separate page) as a separate page 14 at the end of the specification:

--LOW ERUCIC ACID MEADOWFOAM PLANTS AND OILS PRODUCED FROM THEM

ABSTRACT

Low erucic acid mutant meadowfoam (*Limnanthes sp.*) plants were made and identified. The low erucic acid oils of these mutant plants have qualities that make them ideal for cooking, food production, and also as a lubricant. --

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REMARKS

The specification has been amended herein to insert Applicant's claim of priority, and to insert the Abstract, originally included in the first page of the International Application, as the last page of the specification. No new matter has been added by this amendment.

Respectfully submitted,

KLARQUIST SPARKMAN, LLP

By _____
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LOW ERUCIC ACID MEADOWFOAM PLANTS AND OILS PRODUCED FROM THEM

ABSTRACT

Low erucic acid mutant meadowfoam (*Limnanthes sp.*) plants were made and identified. The low erucic acid oils of these mutant plants have qualities that make them ideal for cooking, food production, and also as a lubricant.

LOW ERUCIC ACID MUTANT MEADOWFOAM PLANTS AND OILS PRODUCED FROM THEM

FIELD

The present invention relates to meadowfoam plants and oils produced by these plants.

BACKGROUND

Erucic Acid

Erucic acid is a 22 carbon fatty acid triglyceride with a single double-bond at position 13.

$$CH_3(CH_2)_7CH=CH(CH_2)_{11}CO_2H$$

Erucic acid is found in a number of oil-bearing plants including meadowfoam (*Limnanthes* sp.) and rape (*Brassica* sp.). Erucic acid possesses qualities that make the oil useful industrially, for instance as a lubricant. In the form of its derivative, erucamide, erucic acid is presently used as a renewable industrial oil and a chemical feedstock. The major use of erucic acid is in plastics manufacturing, as a plasticizer and a slip-agent (used in plastic film manufacture to allow sheets of film to slip over each other). Erucic acid is also used in the production of cosmetics, adhesives, pharmaceuticals, surfactants and industrial lubricants. The high thermal stability and high oxidative stability of erucic acid make it particularly useful in industrial applications as an additive to increase thermal and oxidative stability of fuels and industrial oils. High erucic acid rapeseed (HEAR) plants are commonly used as a source of erucic acid for such applications.

Apart from its industrial uses, erucic acid is known to possess useful pharmacological properties especially in the treatment of the debilitating and fatal neuropathic diseases, adrenomyeloneuropathy and adrenoleukodystrophy. In the case of adrenomyeloneuropathy, erucic acid in combination with oleic acid ("Lorenzo's oil") is believed to inhibit the synthesis of very long-chain fatty acids, thereby inhibiting pathology (Aubourg *et al.*, *N. Eng. J. Med.* 329: 745-752, 1993).

Erucic acid-producing plants (especially rapeseed) have been intensively investigated and manipulated to *increase* erucic acid content, and there are over 1000 U.S. patents relating to high erucic acid oils and derivatives. Traditional plant breeding techniques as well as genetic engineering techniques have been used to produce high-erucic acid plants and, in 1995, it was announced that the biotechnology company Calgene had genetically engineered a variety of rapeseed that produces oil with an erucic acid content of up to 90%, about 40% higher that that of typical HEAR plants.

Although erucic acid is useful industrially and pharmacologically, it is harmful to the health of animals (including humans) if consumed in large quantities. Because of this, oil-seed plants that produce oil high in erucic acid cannot be used as a source of oils for cooking or food-production. Early studies indicated that chickens fed a high erucic acid diet developed fatty deposits around their heart. In 1981, hundreds of people died and thousands were poisoned by rapeseed adulterated with aniline which was sold in Spain. The symptoms of the disease, known as "toxic oil syndrome,"

include pulmonary hypertension, ventricular hypertrophy, widespread vascular and neural lesions and other scleroderma-like symptoms. It has been suggested that toxic oil syndrome is caused, at least in part, by erucic acid, possibly in conjunction with other seed-derived oil components (James, *Clin. Cardiol.* 17:463-470, 1994).

Modifying rapeseed plants to *decrease* the amount of erucic acid they produce has long been known and is of considerable economic importance (Stefansson, *High and Low Erucic Acid Rapeseed Oils*, Academic Press, Canada, 1983). In 1958, Canadian scientists began working on the development of rapeseed plants with low erucic acid content, and by a process of selective breeding, succeeded in 1968 in producing a rapeseed plant that produced oil having only about 5% erucic acid. In 1974, the Canadian scientists produced the first commercially useful "double low" cultivar that was low in both erucic and glucosinates (a second toxic component of rapeseed oil). By 1986, plants had been developed that produced oil with less than 2% erucic acid and less than 30 micromoles per gram of glucosinates, and it is this oil that is marketed under the trademark CANOLA™.

Meadowfoam

Meadowfoam (*Limnanthes* sp.) is a relatively unexploited Northern California wildflower from which industrially useful oils may be extracted. Most vegetable oils, such as cottonseed and CANOLA™ oils, contain fatty acid chains of 16 or 18 carbons. Meadowfoam seed, however, contains high percentages of 20- and 22-carbon chain fatty acids. The longer the chain, the more stable the fatty acid making it more heat stable and a better lubricant than shorter chain fatty acids. The seed oil of meadowfoam is a rich source of unique unsaturated long-chain fatty acids (LCFAs). The major fatty acids of meadowfoam seed oil are *cis*-5-eicosenoic (20:1Δ5), *cis*-5-docosenoic (22:1Δ5), *cis*-5 *cis*-13-eicosenoic (22:2Δ5Δ13), and *cis*-13-docosenoic (22:1Δ13) acid. More than 96% of the fatty acids are unsaturated long-chains and *Limnanthes* is one of several known sources of *cis*-5 unsaturated fatty acids (20:1Δ5, 22:1Δ5, and 22:2Δ5Δ13).

The triacylglycerols (TAGs) of meadowfoam oil are unique. Roughly two-thirds of the erucic acid of meadowfoam TAGs are present at the second fatty acid branch of the TAG (*sn*-2) position, while erucic acid is absent from the *sn*-2 position in rapeseed and crambe.

The unique chemical and physical properties of meadowfoam oil are a function not only of unique structure but also of the purity of unsaturated long-chain fatty acids and presence of unique unsaturated fatty acids. These properties have fueled the development of meadowfoam as an industrial oilseed crop.

The oil from meadowfoam is currently used chiefly for lubricant and cosmetic formulation and is found in lipsticks, foundation, and other skin-care products. Meadowfoam oil has several characteristics that would make it ideal for use as a food oil, both for cooking and other food production applications, such as the making of salad oils. These characteristics include very low saturated fat (only one double bond), high thermal stability and high oxidative stability. Additional

economically useful characteristics include high oil yield from the seeds and the low cost of growing the plant.

Despite its very low saturated fat content and high thermal and oxidative stability, meadowfoam oils are presently unusable for food applications because of the unacceptably high concentration of erucic acid in the seed oil. Erucic acid content of wildtype *Limnanthes alba* germplasm typically ranges from about 6.5% to about 18% (Knapp and Crane, *Ind. Crops and Prod.* 4: 219-227, 1995). This erucic acid content is typical of meadowfoam plants. It is believed that no meadowfoam variety in the prior art has seed with a low level of erucic acid, for instance, equal to or less than about 5%.

It would be desirable to produce a low erucic acid meadowfoam plant, the oil of which would be useful for cooking and food production purposes as well as for lubrication purposes.

SUMMARY OF THE DISCLOSURE

The inventors have produced mutant low erucic acid meadowfoam plants that are true breeding. The oil derived from the seeds of these plants contains less than about 5% by weight erucic acid and may be used for cooking, food production and food processing as well as for lubrication purposes. In another embodiment, the seeds of the plant of the invention may contain less than about 3% erucic acid, for instance, about 2.5%, 2%, 1%, 0.5%, 0.1% or no detectable erucic acid.

Seeds of a representative low erucic acid meadow foam plant of the invention have been deposited with the ATCC under deposit number PTA-2338 on August 9, 2000.

The invention embraces whole plants as well as parts of such plants, such as seeds. Also encompassed within the scope of the invention are oils derived from those plants and methods of producing such oils. In one embodiment, the oils provided are food oils. Hybrid plants of the low erucic acid meadowfoam plants of the invention and oils derived from such hybrid plants are also encompassed by the invention.

Yet other embodiments include methods of making and identifying mutant low erucic acid meadowfoam plants. EMS and/or electromagnetic radiation may be used as a mutagen in such methods, for instance. These methods of making and identifying plants of the invention include methods wherein oil content is determined for second generation seeds or alternatively, for third generation seeds. The invention also embraces methods of reproducing low erucic acid meadowfoam plants and reproducing seeds from such plants.

These and other aspects of the invention will become more apparent from the following description.

DETAILED DESCRIPTION

The present invention includes low erucic acid meadowfoam plants that bear seeds which contain a mixture of fatty acids that contains not more than about 5% erucic acid by total weight of fatty acid mixture. The plants of the invention may be referred to as being *low in erucic acid* or as *low erucic acid* plants. The mixture of fatty acids contained in the seed of such plants may be

referred to as *meadowfoam seed oil* or simply *meadowfoam oil*. The erucic acid content of the meadowfoam oil of the invention is not more than about 5% and may be about 4.9%, 4%, 3%, 2%, 1%, 0.5%, 0.1% or less, for instance, below limits detectable by gas chromatography. The oil from the plants of the invention, extracted from the seeds, is low in erucic acid and therefore useful as a food component.

The present invention also includes *hybrid plants* that are the offspring of a cross between a low erucic mutant meadowfoam plant of the invention and another plant. For instance, the mutant meadowfoam of the invention may be crossed with another variety of meadowfoam (*e.g.*, *L. gracilis*, *L. douglasii* or *L. floccosa*) or a related species to create a hybrid plant. Non-related species may also be used, just so long as a fertile plant results from the cross. Such hybrid plants may exhibit low erucic acid characteristics.

Low erucic acid meadowfoam oil may be used for a number of purposes including food processing, preparation and manufacture, and also for lubrication. The meadowfoam mutants of the invention may be made by various mutagenesis techniques including exposure of meadowfoam seeds to chemical mutagens and/or to electromagnetic radiation. Mutagenesis may also be carried out by various molecular biology and genetic engineering techniques. For instance, transposon mutagenesis may be employed to introduce random mutations into the meadowfoam genome.

The invention also includes a method of making and identifying a low erucic acid meadowfoam plant by mutagenizing meadowfoam seeds, growing the seeds to produce seed-bearing plants, harvesting such seeds, planting such seeds to produce a second generation of seed-bearing plants, harvesting the seeds from these plants, measuring the erucic acid content of such seeds and tracing back the parentage of these seeds to identify a plant that produces seeds low in erucic acid. Alternatively, erucic acid content may be measured for seeds produced from first generation plants and the first generation plants identified that produce seeds low in erucic acid. The seeds of one such mutant meadowfoam plant were deposited with the American Type Culture Collection (ATCC, Manassas, VA) on August 9, 2000, under accession number PTA-2338.

A. Abbreviations and Definitions

EMS: Ethyl methanesulphonate
HEAR: High Erucic Acid Rapeseed
ATCC: American Type Culture Collection

Meadowfoam means any plant of the *Limnanthes* species including, but not limited to *L. alba, L. gracilis, L. douglasii, L. floccosa* and all variants of these species including the cultivar "Mermaid".

Erucic acid is a 22 carbon triglyceride fatty acid with a single double bond, having the chemical structure $CH_3(CH_2)_7CH=CH(CH_2)_{11}CO_2H$.

An *oil* is a fatty acid or mixture of fatty acids, generally liquid at about 25° C.

A *food oil* is any edible oil used in the production, preparation or manufacture of food products.

The phrase *percent (%) erucic acid by weight* (in a mixture of fatty acids) refers to a percentage by weight compared to the total weight of fatty acids in a mixture.

The phrase *low erucic acid* or *low in erucic acid* is used to describe a substance or article, usually an oil comprising a mixture of fatty acids, that has an erucic acid content of not more than about 5% by weight. For example, about 5%, 4.9%, 4%, 3%, 2%, 1%, 0.5%, 0.1% or less of the total weight of the oil may be due to the weight of erucic acid.

A *low erucic acid* (mutant meadowfoam plant) is a mutant meadowfoam plant, the seeds of which contain an oil that is not more than about 5% by weight erucic acid. Such seeds may have an erucic acid content of, for instance about 4.9%, 4%, 3%, 2% or 1% by weight. In some cases the erucic acid content of these seeds may be less than 1% by weight, such as 0.5% or 0.1% by weight. Such seeds may even contain no detectable erucic acid.

A phrase referring to a *part of* a plant refers to any plant structure, whether or not currently connected to the plant, including, but not limited to seeds, germplasm, roots, leaves, stems, meristem, sex organ tissues (*e.g.*, anthers, carpels, pistils, pollen, ovaries and flowers) and tissue culture of any plant tissue.

Hybrid plants of a low erucic acid meadowfoam plant of the invention are the offspring of a cross between a low erucic acid mutant meadowfoam plant of the invention and any other plant or plants. Any progeny plant that has in its parental lineage a plant as deposited as ATCC number PTA-2338 (deposited on August 9, 2000) is also a hybrid plant. Such a hybrid plant may be removed from the original parent by many generations and by crosses and back-crosses.

The phrase referring to *oil from* (a particular plant) or *oil derived from* (a particular plant) is used to mean an oil or mixture of oils that has been extracted from the plant in question.

The phrase *extracted from* (a plant), for instance, "a low erucic acid oil *extracted from* (a meadowfoam plant)" refers to a substance, usually an oil, that has been contained in a plant or part of a plant, such as a seed, and that has subsequently been removed from the plant. Thus, for example oil contained in meadowfoam seeds that has been removed from the seeds by mechanical crushing or other means has been *extracted from* the plant.

The word *mutagen* refers to any substance or process that may be used to genetically alter a plant, including, but not limited to chemicals (*e.g.*, EMS or ethylnitrosourea), electromagnetic radiation (*e.g.*, X-rays, gamma rays and alpha particles), and genetic engineering and molecular biology techniques for producing a genetic mutation (*e.g.*, transposon mutagenesis).

The word *mutant* refers to any organism that has been intentionally genetically altered from its native form (*i.e.*, from the form in which it is naturally and generally found in nature).

B. General Methods

1. Plants and Growth Conditions

Any species of meadowfoam may be used to practice the present invention. Meadowfoam plants may be grown under standard horticultural conditions, either in a greenhouse or outdoors, using standard commercial soil mixtures (see Oregon State University extension publication EM8567 (1997) "Growing meadowfoam in the Willamette Valley" by Ehrensing *et al.*). Alternative growing conditions may be used, and soil, temperature, soil moisture and exposure to light may all be adjusted within well known acceptable ranges for growing meadowfoam (and similar) plants. Hydroponic growing methods may also be used to grow the meadowfoam plants. Plants may be pollinated mechanically, for instance, by hand (*e.g.* using an artist's brush) to produce seed. Alternatively, where specific pollination is not vital, plants can be pollinated non-mechanically either through selfing or via other pollination vectors, such as insects.

Seeds may be harvested from mature seed-bearing low erucic acid meadowfoam plants by hand or by standard threshing techniques and such seeds may be used either to produce oil or may be planted under standard conditions described herein resulting in germination and eventual production of mature seed-bearing plants. Where seed is used to produce oil, any known oil extraction technique is appropriate, including but not limited to mechanical crushing of the seeds.

2. Mutagenesis

Mutagenesis may be carried out by subjecting the plant cells to a chemical mutagen, electromagnetic radiation, such as gamma irradiation, or a combination of chemical and electromagnetic mutagens, for a sufficient duration to accomplish mutations that result in the desired lowering of erucic acid content via a genetic modification but insufficient to destroy the viability of the cells and their ability to be germinate and grow into a plant. The desired mutagenesis may be accomplished by use of chemical means such as by contact with, for instance, ethyl methanesulphonate, ethylnitrosourea, etc., or by the use of physical means such as, for instance, x-ray.

Mutagenesis also may be carried out through exposure of plant cells to gamma radiation, such as that supplied by a Cesium 137 source. The gamma radiation may be supplied to the plant cells in a dosage of approximately 60 to 200 Krad., and in some cases, a dosage of approximately 60 to 90 Krad. may be desirable.

Chemical mutagenesis, for instance, using ethyl methanesulphonate (EMS) or ethylnitrosourea, may be used to mutagenize the seeds of a meadowfoam plant. Such procedures generally involve first soaking seeds in distilled water for at least a few hours, generally overnight. Some seeds which possess a particularly impenetrable coating or which are covered in hairs may need to be scarified with sandpaper before soaking. Once rehydrated, the seeds are soaked in a solution of the selected mutagen. The higher the concentration of the mutagen and/or the longer the exposure time, the more likely it is that multiple mutations will be produced and the more likely that the seeds will become non-viable. In some cases it may be desirable to adjust the concentration of

mutagen and exposure time so as to increase the probability of producing one or only a few mutations per seed. By way of example, the concentration of the mutagen EMS used may be between about 0.5 and 0.005 M. The time of exposure may be, for instance, from about 30 minutes to about 48 hours, for instance, from about three hours to seven hours. The mutagen is generally diluted in a buffer, such as KH_2PO_4 to produce a final pH of from about 5.0 to about 9.0; a pH of about 7.0 is commonly used. The length of exposure is dependent to some extent on the concentration of mutagen. The higher the mutagen concentration, the shorter the exposure time needed. Other chemical mutagens that may be used include nitrous acid or various alkylating agents. After exposure to a chemical mutagen, the seeds are generally rinsed or soaked in distilled water, for example, for between one and 24 hours, to remove excess mutagen.

3. Selection of Mutants and Measurement of Erucic Acid Content

Seeds, having been exposed to a mutagen, may be planted out using growing conditions described herein and grown to produce seed. First (M1), second (M2) and third (M3) generations, and so forth, may be planted out and seeds from the M2, M3, and so forth generation analyzed for oil content. The parentage of these seeds may then be traced back to the M2 and M1 generations to identify the low erucic acid mutant plants of interest. Seed from selected plants may be harvested and the seed-oil extracted by extraction into an organic solvent.

An alternate method of extracting oil from seeds is by crushing the seeds in a commercial press, such as a press of the type commonly used for crushing rapeseeds or sesame seeds. Crushing can be used to obtain meadowfoam oils in a quantity convenient for commercial use, for instance, a quantity of at least one liter.

Oil may be analyzed by automated gas chromatography, for instance using a Hewlett Packard 5890 gas chromatograph as described in Example 3, below.

Analysis will allow determination not only of the type of oils in the sample, but also of the quantity of the individual components including the quantity of erucic acid. Plants whose seed oil exhibits low erucic acid content (generally less than about 5% (w/w)) may be selected and bred, for instance by self-crossing (selfing) to establish a true-breeding stock of low erucic acid meadowfoam plants. Such selfing may produce additional plant line generations, for instance fourth generation (M4), fifth (M5) and so on.

Meadowfoam seeds with the desired characteristics may then be multiplied as described herein to produce a uniform plurality of such low erucic acid seeds that can then be used to produce a uniform stand of low erucic acid meadowfoam plants.

4. Seed Deposit of a Representative Low Erucic Acid Meadowfoam Plant

The present invention encompasses a true-breeding, low erucic acid meadowfoam plant and any plant having the characteristics of this plant. Seeds of this plant were deposited with the American Type Culture Collection (ATCC, Manassas, VA) on August 9, 2000, under accession number PTA-2338. The plants of the invention, and seeds from these plants, are also maintained at, and available from, Dr. Steven Knapp at the Department of Crop and Soil Science of Oregon State

University, Corvallis, Oregon 97331. The seeds of this low erucic acid meadowfoam plant contain an oil (*low erucic acid meadowfoam oil*) that is a mixture of fatty acids. This oil contains no more than about 5% erucic acid (w/w).

5. Crosses and Pollination

Crosses may be performed by emasculation of the flower by hand, followed by pollination by hand, for instance, using an artist's brush to transfer pollen to the pistil.

6. Reproduction of Meadowfoam Plants and Production of Hybrid Plants

Viable seeds from low erucic acid meadowfoam plants of the invention may be planted and grown as for any other meadowfoam plant as described to produce mature plants. The low erucic acid characteristics of the plant of the invention may be introduced into other varieties of meadowfoam and into other plants by conventional breeding techniques. For example, pollen may be taken from the plant of the invention and used to pollinate another plant, such as another species of meadowfoam plant. Cross-pollination gives rise to hybrid seeds, which then may be grown to produce mature seed-bearing hybrid plants. The seeds of these hybrid plants may then be analyzed to determine their erucic acid content. In this way, low erucic acid hybrid plants may be produced that combine low erucic acid characteristics with additional advantageous characteristics of other plants, such as low glucosinate levels, high yield and/or disease resistance.

Any meadowfoam plant that is low in erucic acid and that has in its parental lineage a plant as deposited as ATCC No. PTA-2338, may be described as a *hybrid plant* of ATCC No. PTA-2338. For instance, a plant removed by several generations that results from multiple crosses and back-crosses, but which has, as one of its genetic progenitors, the plant deposited as ATCC No. PTA-2338, is such a *hybrid plant.*

Other characteristics of the meadowfoam plants described herein, and particularly of the meadowfoam plant designated ATCC No. PTA-2338, may be modified using traditional plant breeding and genetic engineering techniques. For instance, drought resistance, herbicide resistance and/or disease resistance characteristics may be introduced into hybrid or progeny plants of ATCC No. PTA-2338. This may be done by selective breeding (*e.g.*, by cross-pollination) of ATCC No. PTA-2338 with a plant known to possess the desired characteristic(s) and then selecting a hybrid plant(s) having those characteristics, or by genetic engineering methods. Once introduced, such a trait is fixed in a homogeneous state by continued self-pollination followed by selection and/or haploid production that results in a plant having a substantially uniform phenotype. Additionally, such characteristics may be selected for using a microspore-based selection process wherein embryos are generated from the microspores and subjected to specific conditions, such as the presence of a herbicide, wherein selection for herbicide tolerance is accomplished. Such a technique is described in U.S. Patent No. 5,535,812.

7. Extraction and Storage of Low Erucic Acid Oil.

Low erucic acid oil may be extracted from seeds by crushing of the seeds, for instance by use of a pestle and mortar or a commercial crushing machine. Alternately, oil may also be extracted by the use of a hydrophobic solvent such as hexane. In this method, seed may be ground in a tube containing hexane, the solution evaporated to dryness, and ethyl ether and potassium hydroxide solution in methanol added to release fatty acid methyl esters. Samples from this solution may then be analyzed by gas chromatography using a commercial gas chromatograph machine.

Extracted oil may be stored at room temperature in any suitable container, such as glass or metal or plastic.

The invention also encompasses parts of the low erucic acid meadowfoam plant that may or may not be separated from the rest of the plant. Such parts include, but are not limited to seed, flower, root, stem, leaf, meristem tissue, cultured tissue and sexual organs including carpel, pistil, stigma, stamen, anthers, ova and pollen. The invention also includes oils extracted from the described plants, from hybrid low erucic acid meadowfoam plants and from a part of any such plant.

C. EXAMPLES

1. Mutagenesis of Meadowfoam Seed.

Meadowfoam seeds from the cultivar "Mermaid" (Plant Variety Protection Cert. 8500166) were subjected to EMS mutagenesis.

The seeds were soaked in distilled water for 16 hours at room temperature. The seeds were then soaked in 0.04 M EMS in 0.1 M KH_2PO_4 buffer (pH 7.0) for 5 hours, and then in distilled water for 18 hours to rinse off excess EMS. Seeds were planted and grown in a greenhouse under standard conditions using standard commercial soil mix, without using pesticides.

2. Selection of Low Erucic Acid Meadowfoam Plants

Meadowfoam seeds subjected to mutagenesis as described above were planted to produce 900 mature first generation (M1) seed-bearing plants. These M1 plants produced a second-generation (M2) of seeds. These M2 seeds were harvested and planted to produce seed-bearing M2 plants that produced a third generation (M3) of seeds. Each M3 line traced to a different M2 individual.

Oil was extracted from the M3 seeds and fatty acid content was measured by gas chromatography using samples of 10 seeds per line.

3. Extraction and Analysis of Fatty Acids

Ten seeds per plant were ground by hand in a 16 X 100 mm screw-cap tube containing 1.0 ml of hexane, incubated for 15 minutes at 50° C. Samples were vigorously shaken subsequent to adding 1.0 ml hexane and 0.2 ml 0.15 *M* HCl. A 1.0 µl sample was drawn from the top layer of the mixture and injected into a Hewlett Packard 5890 gas chromatograph (Hewlett-Packard Inc.,

Avondale, CA, USA) fitted with a JW DB-23 (30.0 m X 0.25 mm) fused-silica capillary column (JW Inc., Folsom, CA, USA). The helium gas flow was set at 0.7 ml/min. The column oven temperature was set at 180° C for one minute and increased by 4° C/min to 220° C. The temperature of the injector and flame ionization detector was set at 250° C. Standards from the USDA-ARS (Robert Kleiman, Peoria, IL) were used to identify peaks for 16:0, 18:0, 18:1Δ5, 18:1Δ9, 20:0, 20:1Δ5, 20:1Δ11, 22:1Δ5, 22:1Δ13, 22:2Δ5Δ13, and 24:1.

Results of gas chromatography analysis revealed an M2 line of the plants of the invention having a mean erucic acid content of about 2.5% of the total weight of fatty acid extracted from the seed (see Table 1). Control plants of the parent (Mermaid) were also assayed for erucic acid content (using identical techniques) and were found to have a mean erucic acid content of about 9.4%. This very significant drop in erucic acid content was found to be a genetically stable trait transmitted from generation to generation of mutant meadowfoam plant.

TABLE 1: Fatty acid concentrations for wildtype (WT) and low erucic acid (LE76 M2, M3, M4, and M5) meadowfoam lines.

Line	Fatty Acid (g kg-1)			
	20:1Δ5	22:1Δ5	22:1Δ13	22:1Δ5Δ13
WT	655	34	94	173
LE76 M2	622	25	27	282
LE76 M3	558	29	49	306
LE76-1-11	433	30	13	306
LE76 M4	46	30	33	348
LE76 M5	568	28	29	328

Seeds of the M5 line, derived by selfing from the M2 line originally identified as having a mean erucic acid content of about 2.5% w/w, were deposited with the American Type Culture Collection (ATCC, Manassas, VA) on August 9, 2000, under accession number PTA-2338. Additionally, seeds and plants of this strain are available from Dr. Steven Knapp at the Department of Crop and Soil Science, Oregon State University, Corvallis, Oregon 97331.

4. Exemplary Uses of Low Erucic Acid Meadowfoam Oils

Low erucic acid meadowfoam oils are edible and therefore may be used for cooking and food production and preparation. The high thermal and oxidative stability of these oils makes them very well suited for high temperature cooking such as frying and baking. The low saturated acid content of the oils means that they are a healthy substitute for other cooking oils. Additionally, low erucic acid oils are well suited to industrial lubrication applications because of their high molecular weight and high oxidative and thermal stability. Because of these features, the oils maintain their lubricating properties even under punishing conditions, such as in an internal combustion engine. In addition, the oils of the invention may be used in cosmetics manufacture, such as in the compounding

of lipsticks and foundation creams. The above uses make low erucic meadowfoam oil a very important commodity.

The above embodiments are provided for exemplary purposes only and are in no way meant to narrow the scope of the invention, which should be interpreted in light of the claims. It will be understood by one of ordinary skill in the relevant art that the examples and descriptions of the invention may be modified without departing from the spirit of the invention. We claim all such modifications.

CLAIMS

WHAT IS CLAIMED IS:

1. A meadowfoam plant that produces seed, the seed containing an oil extractable from the seed, the oil having an erucic acid content of not more than about 5% by weight.

2. The plant of claim 1 wherein the erucic acid content of the oil is not more than about 3% by weight.

3. A part of a plant according to claim 1.

4. A seed of a plant according to claim 1.

5. A low erucic acid oil extracted from a plant according to claim 1.

6. A food oil comprising the low erucic acid oil of claim 5.

7. A method of making and identifying a low erucic acid mutant meadowfoam plant, comprising:

contacting meadowfoam seeds with a mutagen;

growing the seeds to produce mature first generation plants bearing second-generation seeds;

harvesting the second-generation seeds from each first generation plant; and

measuring the erucic acid content of at least some of the second-generation seeds, such that at least one low erucic acid mutant meadowfoam plant is identified.

8. A method of making and identifying a low erucic acid mutant meadowfoam plant comprising:

contacting meadowfoam seeds with a mutagen;

growing the seeds to produce mature first generation plants bearing second-generation seeds;

harvesting the second generation seeds from each first generation plant;

planting the second generation seeds to produce a plurality of second generation plants bearing third-generation seeds; and

harvesting the third-generation seeds from each second-generation plant, and measuring the erucic acid content of the third-generation seeds such that at least one low erucic acid mutant meadowfoam plant is made and identified.

9. The method of claim 7 wherein the mutagen comprises ethyl methanesulphonate.

10. The method of claim 8 wherein the mutagen comprises ethyl methanesulphonate.

11. The method of claim 7 wherein the mutagen is gamma radiation.

12. The method of claim 8 wherein the mutagen is gamma radiation.

13 A method of producing a low erucic acid oil comprising:

growing a plant according to claim 1 so as to produce seeds;

harvesting the seeds; and

extracting oil from the seeds.

14. A low erucic acid meadowfoam plant having the characteristic properties of ATCC No. PTA-2338.

15. A part of the plant according to claim 14.

16. Seed of the plant according to claim 14.

17. A hybrid plant of ATCC No. PTA-2338.

18. Seed of the plant of claim 17.

19. An oil extracted from seed of claim 16 or 18.

20. The oil of claim 19 where the oil contains no more than about 5% erucic acid by weight.

21. The oil of claim 19 where the oil contains no more than about 3% erucic acid by weight.

22. A food oil comprising the oil of claim 19.

23. A method of producing seed of a low erucic acid meadowfoam plant, the method comprising:

planting the seed of claim 16 or 18 under conditions that result in the germination of the seed to produce a mature seed-bearing plant; and

harvesting the progeny seed.

24. A method of producing a low erucic acid progeny plant, the method comprising:

crossing a first low erucic acid meadowfoam plant with at least one other plant, wherein the first low erucic acid meadowfoam plant is a plant according to claim 14; and

screening the progeny plants to identify a low erucic acid progeny plant.

25. A low erucic acid hybrid meadowfoam plant produced by the method of claim 24.

26. Seed of the low erucic acid meadowfoam plant of claim 25.

27. A low erucic acid meadowfoam oil, wherein the oil comprises no more than about 5% erucic acid by weight.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
12 April 2001 (12.04.2001)

PCT

(10) International Publication Number
WO 01/24617 A1

(51) International Patent Classification[7]: A01H 5/00, 5/02, 5/04, 5/06, 5/10, 5/12, 1/06, 1/04, C12N 15/01

(21) International Application Number: PCT/US00/23713

(22) International Filing Date: 29 August 2000 (29.08.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/157,256	1 October 1999 (01.10.1999)	US
60/157,258	1 October 1999 (01.10.1999)	US

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/24617 A1

(54) Title: LOW ERUCIC ACID MUTANT MEADOWFOAM PLANTS AND OILS PRODUCED FROM THEM

(57) Abstract: Low erucic acid mutant meadowfoam (*Limnanthes sp.*) plants were made and identified. The low erucic acid oils of these mutant plants have qualities that make them ideal for cooking, food production and also as a lubricant.

TMH:jlb 03/28/02 107953.doc

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COMBINED DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am an original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled LOW ERUCIC ACID MEADOWFOAM PLANTS AND OILS PRODUCED FROM THEM, the specification of which

☐ is attached hereto.

☐ was filed on _____ as United States Patent Application No. _____.

☒ was described and claimed in PCT International Application No. PCT/US00/23713, filed on 29 August 2000.

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I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

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Number	Country	Day/Month/Year Filed	☐ Yes	☐ No

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Application Number	Filing Date
60/157,256	October 1, 1999
60/157,258	October 1, 1999

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